Potential Use of Pyriproxyfen for Control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Perú

MOISÉS SIHUINCHA, ELVIRA ZAMORA-PEREA, WAGNER ORELLANA-RIOS, JEFFREY D. STANCIL,[1] VICTOR LÓPEZ-SIFUENTES,[1] CARLOS VIDAL-ORÉ,[2] AND GREGOR J. DEVINE[3]

Laboratorio Referencial, Dirección de Salud, Iquitos, Perú

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ABSTRACT The effects of pyriproxyfen were tested against a local population of *Aedes aegypti* (L.) in Iquitos, Perú. Bioassays showed that, when applied to late instars, pyriproxyfen prevented adult emergence at extremely low concentrations (LC_{50} = 0.012 ppb). There was no adult emergence from water sampled from storage tanks that had been seeded with the equivalent of 50–83 ppb (AI) pyriproxyfen. Five months after treatment, despite constant dilution of these tanks, water sampled from these sources continued to be lethal to larvae and pupae. Additional studies, carried out in the laboratory, showed that groups of five or 20 female blood-fed mosquitoes, exposed to residues of ≈0.003 g (AI) pyriproxyfen/m^2, could transfer enough chemical to new oviposition sites to prevent ≈80% of adult emergence from larvae developing in that previously uncontaminated water. Moreover, although the fecundity of the adult females used as the transfer vehicles in these tests was unaffected, the subsequent eclosion of the eggs that these mosquitoes laid was decreased by 70–90%. It also was shown that, at very high concentrations (>30,000 ppb), pyriproxyfen-treated water sources were as likely to be used as oviposition sites as untreated sources. These data suggest that treated sites might act as sinks for mosquito reproduction and moreover that such sites might act as dissemination sources for the horizontal transfer of larvicides to new environments by mature females. We review the literature on the environmental and human health effects of this compound and discuss its potential for use as a mosquito control agent in the field.

KEY WORDS pyriproxyfen, *Aedes aegypti*, mortality, fecundity, horizontal transfer

Aedes aegypti (L.) (Diptera: Culicidae) is a peridomestic mosquito species that exhibits a diurnal, bimodal feeding pattern in Iquitos, Perú. Adults are commonly found in secluded areas of a dwelling, such as underneath beds and resting on clothes in the lower portions of closets. Oviposition and larval development often occurs in artificial containers such as flower pots, cisterns, water jars, and other storage vessels (Morrison et al. 2004). Throughout the tropical and semitropical world, *Ae. aegypti* is the predominant vector of dengue; a mosquito-borne arbovirus belonging to the family Flaviviridae capable of causing dengue fever, dengue hemorrhagic fever and dengue shock syndrome (Gubler and Kuno 1997).

Iquitos is an urban area located in the eastern part of the Department of Loreto in the Peruvian Amazon. It has an estimated 350,000 inhabitants. The majority of houses do not have constant access to running water (although ≈77% of households have piped water available for at least a few hours per day). Consequently, houses often contain one or more of the following water storage systems; concrete reservoirs of up to 1000 liters, 55 U.S.-gal drums (≈210 liters), and large metal tanks. These sites, and a plethora of rubber tires, pots, and disposed plastic and tin containers, provide ideal breeding sites for *Ae. aegypti* (Schneider et al. 2004). During 1990, this town was the center of the first (laboratory-confirmed) dengue epidemic in Perú. The last severe Peruvian outbreak was in 2001, when 23,304 cases were reported countrywide (OPS 2004). The need for effective *Ae. aegypti* control is thus clear and urgent.

Pyriproxyfen is classified as a juvenile hormone (JH) analog and has been used against a range of arthropods since its introduction to the agrochemical market in the early 1990s. The World Health Organization has recently recommended that it be used for the control of some mosquito species (WHO 2001).

The opinions and assertions expressed herein are those of the authors and do not necessarily reflect the official policy or position of the U.S. Departments of the Navy or Army, the U.S. Department of Defense, the U.S. Government, or any other organization listed.

[1] Naval Medical Research Center Detachment, United States Navy, Lima, Perú, Unit 3800, APO AA 34031.

[2] Dirección de Salud (DISA), Región Loreto, Iquitos, Perú.

[3] Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden AL5 2JQ, United Kingdom. Address while on sabbatical: c/o Paula Maguina, Asociación Benéfica PRISMA, Carlos Gonzáles 251, Urb. Maranga, Lima 32, Perú.

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The mode of action of pyriproxyfen is not known, and the molecule bears little resemblance to endogenous insect JH, but it affects JH and ecdysteroid titers in a variety of arthropods (Hatakoshi et al. 1986, Zufelato et al. 2000) and is a potent inhibitor of embryogenesis, metamorphosis, and adult formation (Ishaaya and Horowitz 1992). Over the past 20 yr, several studies have been published that specifically examine the utility of pyriproxyfen as a valuable tool for the control of the yellow fever and dengue vector, *Ae. aegypti.* In general, there is a consensus that, as a result of its larvicidal and pupacidal action, pyriproxyfen is effective at inhibiting adult emergence in this species at concentrations ≤1 ppb (Estrada and Mulla 1986, Hatakoshi et al. 1987, Loh and Yap 1989, Itoh 1994, Satoh et al. 2003).

Pyriproxyfen is not an adulticide nor are there many reports on any direct ovicidal effect in mosquitoes (but see Vasuki 1990). In addition to its larvicidal activity, it has been reported as decreasing the fertility and fecundity of *Ae. aegypti* adults that develop from sublethally exposed larvae (Loh and Yap 1989, Dash and Ranjit 1992), and this effect seems to extend to adults that have been directly treated with pyriproxyfen (Itoh 1994). There is an increasing amount of evidence that suggests that adult mosquitoes, which are not killed by exposure to pyriproxyfen, can act as vehicles for the dissemination of pyriproxyfen to previously uncontaminated environments. Once in those environments, the tiny doses of pyriproxyfen that are transferred can affect the development of previously unexposed larvae (Itoh 1993, 1994).

Pyriproxyfen also shows considerable potential for the control of *Ae. aegypti* when applied to water storage vessels under field conditions (Itoh 1993, Nayar et al. 2002). A variety of field studies on other species reinforce this potential (Chavasse et al. 1995 for *Culex quinquefasciatus* Say; Yapabandara et al. 2001, Yapabandara and Curtis 2002 for *Anopheles culicifacies* (Giles) and *Anopheles subpictus* (Grassi); Lee 2001 for *Aedes togoi* (Theobald)).

As a prelude to proposed field studies, we present the results of laboratory investigations that confirm some of the observations published by others and provide more detail on additional factors that might influence the efficacy of pyriproxyfen as a control tool for Peruvian populations of *Ae. aegypti.* We provide a comprehensive examination of the effects of a granular formulation of pyriproxyfen on *Ae. aegypti* originating from the field. We report on the effects of this juvenile hormone analog on 1) adult emergence from exposed fourth instars and pupae, 2) egg eclosion, 3) the use of adults as vehicles for the transfer of lethal doses of larvicide to previously untainted oviposition sites, 4) the effect of adult exposure on fertility and fecundity, 5) the repellency of pyriproxyfen to ovipositing adults, and finally 6) the residual effects of pyriproxyfen applied to large storage tanks in the field. We also present a comprehensive discussion on the human health and environmental repercussions of using this control tool.

Methods and Materials

All experiments were conducted at the Laboratorio Referencial, Dirección de Salud, Iquitos, Perú, between May and July 2004 (except for studies on water tanks, which were carried out between February and July 2004). The *Ae. aegypti* colony was maintained in the laboratory (photoperiod of 12:12 [L:D] h, 30 ± 4°C, 48% RH). Under these conditions, the approximate time taken for 50% of successfully eclosing eggs to reach adulthood was 13 d (unpublished observations).

Blood-fed adults were maintained in large polystyrene/gauze cages with sugar solution provided ad libidum. These cages contained disposable plastic cups of tap water lined with filter paper for oviposition. Eggs were removed at regular intervals and air-dried for subsequent use (the eggs of *Ae. aegypti* can survive for several months in this desiccated state; Sota and Mogi 1992). When required, those eggs were hatched in enameled trays containing an infusion of hay and water (≈10 g hay/1 liter of water boiled for ≈5 min and allowed to cool to 35°C before being added to the trays). Resulting larvae were maintained in trays of water with pulverized fish food, TetraMin (Tetra Werke, Melle, Germany). Pupae and some of the water in which they were developing were collected in cups and returned to the large polystyrene/gauze cages to emerge, mate, blood-feed, and continue the rearing cycle. The colony was regularly augmented by pupal collections taken from Iquitos and surrounding areas.

All experiments were conducted using the pyriproxyfen formulation Sumilarv 0.5 G (Sumitomo Chemical Co., Osaka, Japan). This is a granular formulation of 0.5% (AI) (wt:wt) (5000 ppm). To minimize potential contamination of experiments with minute doses of pyriproxyfen, all materials used for containing eggs, larvae, or adults over the course of the experiments were disposed of after each test. Care was taken to ensure that all manipulation of eggs, larvae, or adults was undertaken using dedicated and disposable plastic pipettes.

Effects on Late Instars. The granular formulation of pyriproxyfen was pulverized to the consistency of talcum powder with a mortar and pestle and agitated for 1 h in tap water. This suspension was used to derive final concentrations of 0.0005, 0.005, 0.05, and 0.5 ppb in tap water (calculated on the assumption that the entire active content of the formulation had entered solution). Batches of 25 fourth instars were added to 1-liter disposable pots containing 250 ml of the abovementioned solutions and ≈0.01 g of TetraMin as a food source. Controls consisted of tap water and TetraMin only. All pots were capped with gauze to prevent the escape of emerging adults and were monitored for 10 d. This was sufficient time to allow 100% of all larvae and pupae to die or to emerge successfully as adults. Each day, molted exoskeletons, dead larvae or pupae, and emerged adults were removed. Each concentration and the control were represented by four replicates, and the entire assay was repeated three times.

Cumulative totals of dead larvae and pupae from each assay were derived, and data were pooled for dose–response analysis by probit (by using the probit analysis package Polo Plus, LeOra Software, Berkeley, CA).

Effects on Eggs. Batches of 30–60 eggs (2–7 d old) were cut from dried filter paper under a binocular microscope and exposed to pyriproxyfen concentrations in a hay/water infusion (see above). Preliminary experiments showed that the concentrations that affected late instars had no effect upon egg eclosion. In subsequent experiments, therefore, a series of greater concentrations were prepared (250, 1,250, 6,250, and 31,250 ppb) in 200 ml of hay-infused water. The number and percentage of successfully eclosed eggs was monitored after the 3 d by removing the filter paper and counting the number of eclosed and uneclosed eggs under a binocular microscope. Four replicates represented each dose, and the experiment was conducted twice. Results were recorded in terms of percentage eclosion, and the data were arcsine transformed and subjected to analysis of variance (ANOVA) for statistical comparison.

Adults as Vehicles for Horizontal Transfer of Pyriproxyfen. The granular formulation of pyriproxyfen was pulverized with a mortar and pestle to the consistency of talcum powder and agitated for 1 h in acetone. The ratio of pyriproxyfen formulation to acetone was 1 g of pulverized formulation (5 mg [AI]) to 10 ml of 100% acetone. This suspension, newly agitated, was then used to coat 250-ml Wheaton media bottles (67 by 152 mm) at the rate of 200 μl (0.1 mg [AI]) per bottle. An additional 1 ml of 100% acetone was added to the bottle to facilitate uniform coating of the bottles. The bottles were rolled in all planes until both they and their caps were evenly coated with pulverized pyriproxyfen. The approximate inner surface area of the bottles was 370 cm^2, which resulted in an approximate coverage of 0.003 g (AI)/m^2 (10,000 cm^2). In total, nine bottles were coated in this manner (3 by 3 treatments), and an additional three bottles (controls) were coated with acetone alone. Groups of 20 or five blood-fed female mosquitoes (fed on the same day as they were exposed) were confined to these bottles for 2 h, 1 h, and 30 min. Control mosquitoes were confined to acetone-treated bottles for 2 h. Bottles were turned every 15 min to maximize the chances of mosquitoes picking up the pulverized material. After confinement, the group of mosquitoes in each bottle was removed and transferred to corresponding 1 liter pots containing 200 ml of clean water and 25 uncontaminated fourth instars (with TetraMin added as a food source). These pots also were lined with filter paper as a substrate for oviposition, and a cotton pad of 5% sugar solution was placed on the gauze cap as a food source for the newly transferred adults. These pyriproxyfen-exposed adults were confined to these pots for 3 d and then removed. The number of surviving adults was noted, as was the number of dead late instars or newly emerged adults.

The oviposition substrate itself (the filter paper), which held the majority of oviposited eggs, was removed and placed in a new, clean pot with 200 ml of a water/hay infusion for subsequent observation of eclosion (see below). The original pots containing the larvae were maintained for a further 7 d. This was sufficient time to allow 100% of all larvae and pupae to die or to emerge successfully as adults. Each day, molted exoskeletons, dead larvae or pupae, and emerged adults were removed. Each treatment and the control were represented by three replicates, and the entire assay was repeated three times. Data were arcsine transformed and subjected to ANOVA for statistical comparison.

Effects of Adult Exposure on Fertility and Fecundity. The eggs that were laid during the course of the experiments on the horizontal transfer of pyriproxyfen (see above) were maintained in clean pots in a hay infusion for 3 d. After this time, the papers were removed, and the total and uneclosed numbers of eggs were counted. The results were recorded in terms of proportion eclosed, and the data were arcsine transformed and subjected to ANOVA for statistical comparison.

Repellency of Pyriproxyfen-Treated Water to Ovipositing Females. Three large polystyrene/gauze cages (1 m^3) were used to house three replicates of simple choice experiments. Each cage contained two 1-liter pots. One contained 200 ml of a 31,250 ppb (AI) pyriproxyfen solution in tap water. The other contained 200 ml of tap water alone. Both these pots were lined with filter paper as an oviposition substrate. A smaller pot containing cotton soaked in a 5% sugar solution was placed between these two pots. Thirty female mosquitoes, blood-fed on the same day as the experiment was carried out, were introduced to each cage. After 3 d, these pots were removed, and the number of eggs laid in each was counted. The entire experiment was repeated three times. The experiment was also repeated using a hay infusion instead of simple tap water. For each water type (tap water or hay infusion), the data were pooled, and differences between observed and expected outcomes were analyzed using 2 by 2 contingency tables.

Efficacy of Pyriproxyfen Treatment in the Field. In total, 16 water tanks were identified for these experiments. Tanks were chosen on the basis that they were at risk of acting as breeding sites and therefore only tanks that contained *Ae. aegypti* larvae before treatment were selected. The volume of those tanks chosen was 200, 300, and 600 liters. These were contained within 16 individual households in the town of Iquitos, Loreto, Perú, and were in constant use as water sources for washing and bathing. They were not used as drinking water sources. The tanks were treated at the rates of 2 g of formulation/200 liters, 4 g/300 liters, and 10 g/600 liters as recommended by the manufacturer (equivalent to 50, 67, and 83 ppb [AI]). Pyriproxyfen was applied in a gauze bag, suspended in the body of the tank by a wire. Each month, for 5 mo, liter samples of this water were collected and returned to the laboratory. Here, batches of 25 laboratory-reared fourth instars were added. These were compared with control pots containing 1 liter of uncontaminated tap

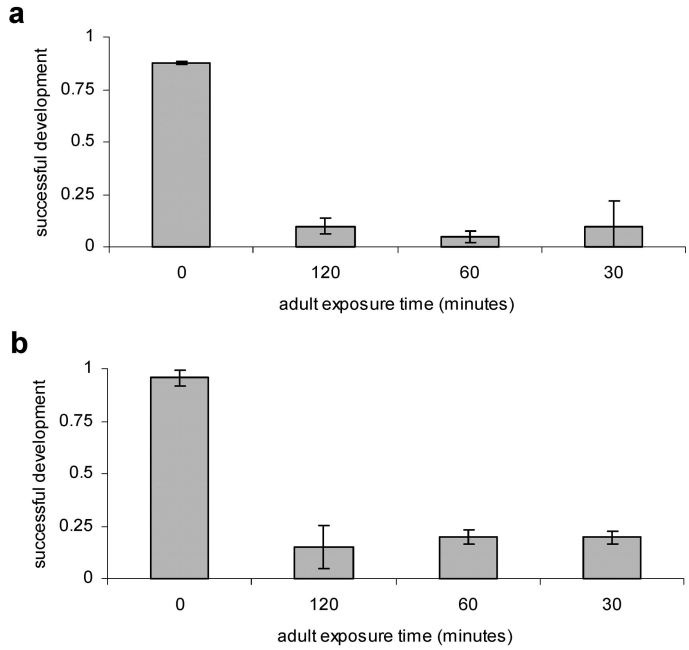

Fig. 1. (a) Adult females as vehicles for insecticide transfer: effects of 20 exposed adults on the successful development of larvae in previously untreated water (mean fraction developing and 95% CL). (b) Adult females as vehicles for insecticide transfer: effects of five exposed adults on the successful development of larvae in previously untreated water (mean fraction developing and 95% CL).

water. TetraMin was added to all pots as a food source. The number of larvae and pupae that had died over a 6-d period was noted and expressed as percentage mortality. For statistical comparison, data were arcsine transformed and subjected to ANOVA.

Results

Effects on Late Instars and Pupae. The LC_{50} that prevented adults from emerging from fourth instars and pupae developing in pyriproxyfen-treated water was 0.012 ppb ($n = 1151$; 95% CL, 0.005–0.021; slope, 1.12). The LC_{90} was 0.61 ppb (95% CL, 0.098–0.310).

When analyzed separately, the three replicates returned LC_{50} values of 0.004, 0.014, and 0.024 ppb (95% CL, 0.0009–0.01, 0.005–0.03, and 0.008–0.04 ppb, and slopes of 0.95, 1.1, and 1.7, respectively). There were no significant differences between these values, suggesting that the methodology used was robust and could be used by other laboratories interested in collecting baseline data for this pyriproxyfen formulation.

Effects on Eggs. All doses permitted >90% eclosion of eggs. The percentage of eggs remaining unhatched after 3 d at 0, 250, 1,250, 6,250, and 31,250 ppb was 6.0, 6.5, 3.8, 6.5, and 7.3%, respectively. There were no significant differences between these values ($F = 0.68$, df = 4, $P = 0.61$).

Adults as Vehicles for Horizontal Transfer of Pyriproxyfen. When adult blood-fed females are exposed to approximate residues of 0.003 g (AI)/m^2, they can clearly transfer enough pyriproxyfen to untreated sites to prevent larvae therein from developing to adulthood (Fig. 1a and b). When groups of 20 females were exposed to pyriproxyfen and then transferred to uncontaminated pots containing healthy larvae, only 8–15% of those larvae emerged as adults. This was in stark contrast with control pots, where 87% of larvae developed to adulthood ($F = 46.4$, df = 3, $P < 0.0001$). There were no significant differences in larval survival related to the length of adult exposure (120, 60, and 30 min; $F = 0.49$, df = 2, $P = 0.62$).

When groups of five females were exposed to pyriproxyfen and then transferred to uncontaminated pots containing healthy larvae, 20–22% of those larvae emerged as adults. In the control pots, 93% of larvae developed to adulthood ($F = 25.9$, df = 3, $P < 0.0001$). Again, there was no relationship between the exposure time of the adults and larval survival ($F = 0.14$, df = 2, $P = 0.87$).

It is of interest that the experiments using groups of five females for chemical transfer were associated with greater larval emergence than those using 20 females. This suggests, unsurprisingly, that larger groups of females can transfer more pyriproxyfen than smaller groups of females. However, in this instance, two-sample *t*-tests assuming equal variances did not reveal any significant differences for any given exposure time between the groups of 20 or five females (two-tailed tests, $P = 0.06–0.61$).

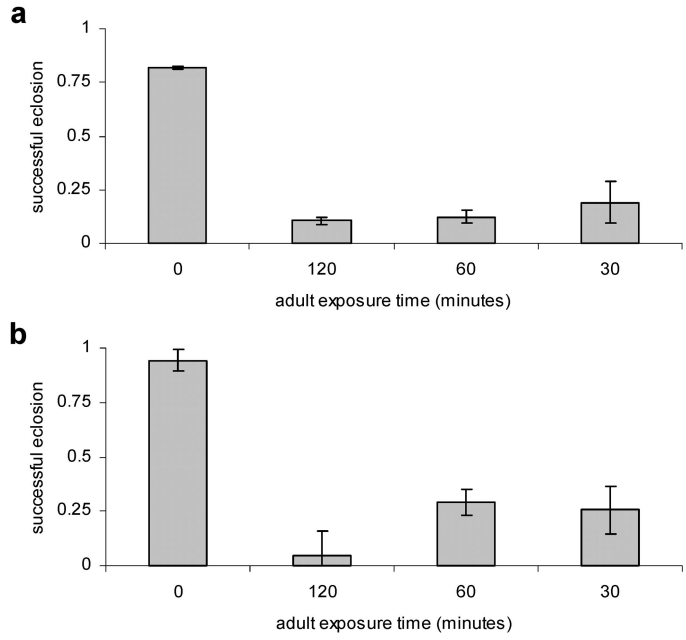

Fig. 2. (a) Effect of adult exposure on fraction of eggs eclosing: effects on groups of 20 adults (mean fraction eclosing and 95% CL). (b) Effect of adult exposure on fraction of eggs eclosing: effects on groups of five adults (mean fraction eclosing and 95% CL).

Effects of Adult Exposure on Fertility and Fecundity. When expressed as the number of eggs laid per surviving female (regardless of group size; five or 20 females), there was no difference between the treatments (10.7–15.9 eggs per surviving female; $F = 0.42$, df = 7, $P = 0.88$).

Of the experiments involving groups of 20 females, 81% of eggs laid by unexposed insects eclosed successfully. This contrasted greatly with the fact that only 12–20% of eggs laid by exposed adults hatched ($F = 38.9$, df = 3, $P < 0.0001$) (Fig. 2a). In the experiments involving groups of five females, 91% of eggs laid by unexposed females eclosed in comparison with 11–31% of eggs laid by exposed females ($F = 17.8$, df = 3, $P < 0.0001$) (Fig. 2b). There was no significant variation in eclosion between the different pyriproxyfen treatments (120-, 60-, or 30-min exposure) for either experiments involving 20 females ($F = 0.48$, df = 2, $P = 0.62$) or five females ($F = 2.66$, df = 2, $P = 0.10$).

Repellency of Pyriproxyfen-Treated Water to Ovipositing Females. There were no differences between the number of eggs laid per female in pyriproxyfen-treated tap water and in untreated tap water (mean ± SE, 3.3 ± 0.52 and 3.7 ± 0.69, respectively). Nor were there differences in numbers laid in the treated and untreated hay infusions (11.5 ± 3.0 and 10.9 ± 2.6, respectively). It was clear that even at extremely high doses, pyriproxyfen had no effect on the number of eggs deposited (see statistics in Table 1).

The presence of organic material in the water greatly increased the number of eggs that were oviposited (Table 1). In two-tailed *t*-tests assuming equal variances, $P = 0.045$ for a comparison between non-pyriproxyfen-treated media and $P = 0.049$ for a comparison between pyriproxyfen treatments.

Efficacy of Pyriproxyfen Treatment in the Field. Before treatment, all 16 tanks chosen for the study contained *Ae. aegypti* larvae and pupae (4–68 individuals; mean ± SD, 32.3 ± 22.8). During the course

Table 1. Oviposition in pyriproxyfen-treated and untreated water sources

Water type	Pyriproxyfen treatment	No. of eggs deposited (pooled totals)	Ratio	χ^2 and *P* values in comparison with expected
Tap water	Present	594	1:1.13	$\chi^2 = 2.4$, df = 1, $P = 0.12$
	Absent	672		
Hay infusion	Present	3,109	1:0.95	$\chi^2 = 1.98$, df = 1, $P = 0.16$
	Absent	2,954		

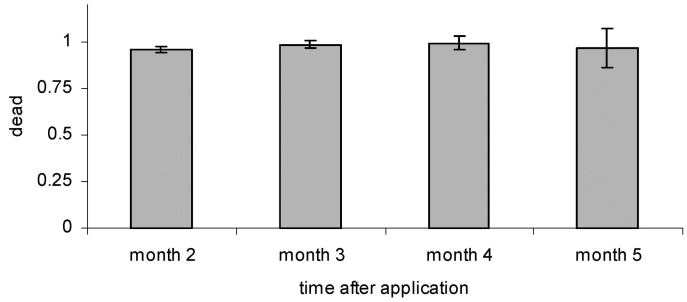

Fig. 3. Larval/pupal mortality in water samples taken from pyriproxyfen-treated tanks (mean fraction dying and 95% CL).

of the experiment, the number of water sources sampled deviated from 16 because of difficulty in gaining access to households (therefore $n = 8–13$). Over the 5-mo period, tap water controls did not yield a single dead larva or pupa. In comparison, water collected from tanks treated with 50–83 ppb remained effective at almost completely inhibiting adult emergence for the entire 5-mo period (88.0–96.0% mortality; Fig. 3). This was clearly extremely significant compared with the zero mortality seen in the controls ($F = 7.4$, df = 4, $P = 0.0002$), but there were no differences in efficacy between months ($F = 0.53$, df = 3, $P = 0.66$).

Discussion

The current larvicide used in Iquitos for *Ae. aegypti* control is the organophosphate temephos. It is currently added to water storage tanks at the rate of 100 g of a 1% (wt:wt) granular formulation/1000 liters (1000 ppb [AI]). It is a relatively safe compound and has been used for the control of *Ae. aegypti* around the world for decades. In West Africa, it is used at the same rate in drinking water for the control of the copepods (*Cyclops* spp.) that act as secondary hosts for the guinea worm *Dracunculus medinensis* (L.) (Sam-Abbenyi et al. 1999). Despite the fact that it is a mainstay of such vector control programs, there are several factors that threaten the use of temephos. First, it is an acetylcholinesterase inhibitor, thus prolonged exposure, accidental ingestion, or misapplication can be hazardous (Brown and Brix 1998, Blain 2001), and acetylcholinesterase inhibitors are currently being phased out of pest control programs in the United States and Europe. Second, there are even suggestions that temephos may be genotoxic at the dose used for *Ae. aegypti* control, which would make it hazardous in drinking water (Fortes Aiub et al. 2002). Third, at its recommended field rates (≈50–500 ppb, assuming volumes of water 0.5 m in depth; Anonymous 1998), temephos would seem to present considerable environmental risk to fish and aquatic invertebrates compared with other mosquito larvicides (Pierce et al. 2000; Brown et al. 1996, 2002). This is a major concern given that many breeding sites for *Ae. aegypti* are found outside houses in gardens and yards (Morrison et al. 2004). Finally, resistance to temephos by *Ae. aegypti* is widespread in South America (Periera Lima et al. 2003, Aparecida Braga et al. 2004), and there are reports of cross-resistance to the pyrethroids, which, at times of dengue outbreaks, are used to fumigate houses and reduce adult mosquito populations (Rodriguez et al. 2002). These are all factors that make the identification of a new, safe, unresisted larvicide with long-lasting residual activity of the greatest importance.

Laboratory-Derived Data. Pyriproxyfen is undoubtedly one of the most effective larvicides available against *Ae. aegypti.* Estrada and Mulla (1986) found that the EC_{95} value that prevented fourth instars of *Ae. aegypti* from emerging as adults was 2.6 ppb. Hatakoshi et al. (1987) reported that it was more active than methoprene, diflubenzuron, or temephos against last instars of *Ae. aegypti* and reported an LC_{50} value of 0.023 ppb. Loh and Yap (1989) reported an EC_{50} value of 0.214 ppb. Itoh (1994) reported that the LC_{50} for two larval populations of *Ae. aegypti* ranged from 0.011 to 0.056 ppb. Satho et al. (2003) determined that 1 ppb of technical grade pyriproxyfen dissolved in ethanol prevented 58–86% of female emergence but that 0.01 ppb inhibited 0–11% of females from emerging. The differences in efficacy that are reported by the above-mentioned studies are presumably the result of differences between strains, formulations, and experimental conditions, but it is clear that there is a consensus that pyriproxyfen is effective at inhibiting adult emergence at concentrations of ≤1 ppb. Our results show that, against a Peruvian population of *Ae. aegypti*, pyriproxyfen was effective at the lower end of the range reported for this compound (≈0.01 ppb). Pyriproxyfen is several hundredfold more toxic against this species than other commonly cited larvicides such as temephos (LC_{50} = 2–20 ppb; Pereira Lima et al. 2003) or *Bacillus thuringiensis* (Berliner) (Bt) (LC_{50} = 10–60 ppb; Amalraj et al. 2000, Blanco et al. 2002).

Pyriproxyfen is also effective against other mosquito species. Ali et al. (1995) showed that, against *Aedes albopictus* (Skuse) larvae, and in comparison

with organophosphates (including temephos), pyrethroids, and *B. thuringiensis*, pyriproxyfen was more toxic by an order of magnitude (LC_{90} = 0.4 ppb). Nayar et al. (2002) looked at granular formulations of pyriproxyfen against laboratory-reared larvae of *Ae. aegypti*, *Ae. albopictus*, *Ae. taeniorhynchus* (Wiedemann), *An. quadrimaculatus* (Say), and *Culex nigripalpus* (Theobald). Applied at 20 and 50 ppb (AI), it inhibited (>80–100%) emergence in the laboratory and the field. In all species, pyriproxyfen induced complete inhibition of adult emergence for several weeks after treatment, even at the lower rate of 20 ppb. Chism et al.(2003) found that pyriproxyfen was effective at 2 ppb against third and fourth instars of *Ae. albopictus* and at just 0.03 ppb against *Ochlerotatus triseriatus* (Say).

Pyriproxyfen has been shown to have limited effects on eggs. Vasuki (1990) exposed eggs (0–1 and 12–18 h old) to concentrations of 0.1 to 1000 ppb pyriproxyfen. At 1000 ppb, 90% of *Ae. aegypti* eggs were inhibited from hatching. Our data show, however, that for eggs >2 d old, there is no inhibitory effect upon eclosion, even at >30,000 ppb.

Pyriproxyfen also can have transovariole effects on mosquitoes. Itoh et al. (1994) found that it affected egg maturation of blood-fed females. The number of eggs deposited decreased concurrently with the number of days before the bloodmeals that the adult mosquitoes received treatment (i.e., the effect was greatest for those females that were blood fed on the same day as exposure; Itoh et al. 1994). We also found that when groups of adults were exposed on the same day as they were blood fed, the successful eclosion of eggs laid subsequently was drastically decreased. The residues of 0.003 g (AI)/m^2 that we used were >300-fold lower than those used by Itoh et al. (1994).

Field Data. In our field studies, conducted on water storage tanks in Iquitos, initial concentrations of 50–83 ppb (assuming all the available formulation was in solution) were effective at preventing eclosion for 5 mo. These tanks were in constant use and even assuming modest turnovers of liquid by householders, the concentration of pyriproxyfen in those tanks after a 5-mo period would be several orders of magnitude lower than those present on initial treatment. This level of efficacy is in marked contrast to the residual effects of alternative treatments such as *B. thuringiensis* (sometimes effective for just a few days; Amalraj et al. 2000) or temephos (effective for weeks rather than months; Yapabandara and Curtis 2002).

Our results reflect those of other studies. Kawada et al. (1988) tested a soluble emulsifiable concentrate, a wettable powder, and a granule under laboratory and field conditions and determined that the granular formulation was the more effective. Itoh (1993) looked at a slow-release formulation of pyriproxyfen for *Ae. aegypti* control in water storage jars that were constantly being used and replenished. An amount that would be equivalent to 200 ppb was used to treat containers that were emptied (the formulation remaining) and refilled at 7-d intervals. After four consecutive replenishments, the formulation still gave >90% kill of larvae. Nayar et al. (2002) examined the effectiveness in the laboratory and field of the granular formulation Sumilarv 0.5 G on a variety of mosquitoes, including *Ae. aegypti*. Pyriproxyfen was effective for the entire 6-wk test period, whether in the laboratory or in 100 liter tanks held outdoors (20 and 50 ppb).

In addition to these highly artificial studies, there are reports that consider pyriproxyfen's efficacy under difficult field conditions. For example, Chavasse et al. (1995) showed that when areas of flooded land and blocked drains were treated with 100 ppb pyriproxyfen, emergence of *Cx. quinquefasciatus* adults was inhibited for 4 wk during the rainy season and for up to 11 wk during the dry season. Yapabandara and Curtis (2002) looked at the use of pyriproxyfen to control malaria vectors *Anopheles culicifacies* and *Anopheles subpictus* in hand-dug pits in Sri Lanka. Doses of 100 ppb completely inhibited emergence. Pyriproxyfen only required reapplication twice a year, whereas temephos required 12 applications per year. Further studies by this team (Yapabandara et al. 2001) showed that pyriproxyfen intervention caused significant reductions in the adult populations of *An. culicifacies* and *An. subpictus* and that it reduced the incidence of malaria in the intervention villages by ≈75%. Prevalence of parasitaemia also declined significantly. In addition to the drainage and stagnant waters referred to above, Lee (2001) noted that a granular formulation of 0.5% pyriproxyfen inhibited emergence of *Ae. togoi* in brackish water in rock pools. Adult emergence was completely inhibited in fourth instars and pupae from 5 to 40 d after treatment with 50 ppb. Lee (2001) further suggested an effective dose for long-term *Ae. togoi* control of 0.05–0.1 mg/liter of 0.5% pyriproxyfen granules. In terms of active ingredient, that is the equivalent of 0.5 ppb.

Horizontal Transfer by Exposed Adults and Effects on Fecundity and Fertility. Itoh et al. (1994) showed that blood-fed female *Ae. aegypti* exposed to a pyriproxyfen residue of 1.0 g (AI)/m^2 for 30 min and then allowed to lay eggs in water containing fourth instars could affect the subsequent adult emergence of those immatures. Transmission of pyriproxyfen from the females to the water was thus proven. Chism et al. (2003) also found that that gravid females exposed to surfaces treated with pyriproxyfen (at 2.0–4.0 g [AI]/m^2) for 1 h and then allowed to oviposit in larval "microcosms" could transfer effective larvicidal doses to these new environments. Densities of five treated females per new site resulted in ≈70% inhibition. They suggested that the use of pyriproxyfen-treated oviposition containers could be used to encourage horizontal transfer of pyriproxyfen to mosquito oviposition sites. Our results also showed this effect, from an exposure of just 30 min to only 0.003 g [AI]/m^2. The amount of pyriproxyfen that could be transferred (as assessed by the subsequent mortality of the larvae confined to the new oviposition site) was not dependent upon length of exposure.

It is possible that such effects could be manipulated to increase the efficacy of pyriproxyfen appli-

cation. This would be dependent on whether pyriproxyfen-treated sources were also repellent. It is clear that not all insecticides affect oviposition preference (e.g., methoprene; Reiter et al. 1991) and that the attractancy of sites can be improved by the addition of attractants (e.g., hay infusions; Beehler and Mulla 1993). In our study, we established that *Ae. aegypti*, when given a choice, show no preference for ovipositing in nontreated water over pyriproxyfen-treated water. This was despite the extremely high concentration of pyriproxyfen present (>30,000 ppb). This would be important were we to consider developing oviposition traps or resting traps for the exposure of adults. Moreover, it suggests that treated containers will act as reproductive "sinks" for *Ae. aegypti*. Casual observations during the course of our trials with storage tanks showed that *Ae. aegypti* continued to develop in treated water tanks despite the fact that this water was still effective at inhibiting adult emergence. Itoh (1993) also assessed the utility of manipulating adults for the dissemination of pyriproxyfen. Adult resting traps made of black mesh and coated in pyriproxyfen were set up in a house containing several oviposition pots. Those pots were then seeded with larvae to check for the horizontal transfer of pyriproxyfen (by observing the inhibition of larval emergence). Inhibition of emergence was observed although the experiment seemed to be poorly controlled.

Parallels for this method of pest control include the work of Schlein and Pener (1990) who noted that *Culex pipiens* (L.) could transfer *B. thuringiensis* toxins in sugar baits to distant oviposition sites. Many other studies have noted that cockroaches can be used to transfer treated baits to otherwise inaccessible breeding sites (Kopanic and Schal 1999, Buczkowski et al. 2001, Buczkowski and Schal 2001).

Implications of Pyriproxyfen Use in the Field. The WHO (2001) has classified pyriproxyfen as "unlikely to present acute hazard in normal use." It states that "pyriproxyfen does not pose a carcinogenic risk to humans" and that "pyriproxyfen is not genotoxic." From a health standpoint, therefore, it is an exceptionally safe compound. As a result of its efficacy, World Health Organisation Pesticide Evaluation Scheme has recommended the use of pyriproxyfen for mosquito control (WHO 2001). Due to its negligible mammalian toxicity, a Food and Agriculture Organization (FAO)/WHO working group also determined that it was safe to add to drinking water at the rate of 10 ppb (FAO 2001). The WHO has subsequently suggested that, based on an allocation of 10% of the allowable daily intake (ADI) to drinking water; a value of 300 μg/liter would be acceptable. This is the equivalent of 300 ppb (WHO 2002). This value is orders of magnitude greater than that which would be needed to be effective for *Ae. aegypti* control (see above).

The Environmental Protection Agency (EPA) considers pyriproxyfen to be a relatively safe chemical in terms of its mammalian health effects and to have a favorable environmental profile (hence its classification by the EPA as a reduced-risk pesticide (http://www.epa.gov/pesticides/health/reducing.htm). When reviewing laboratory and field-derived data on the risks posed by pyriproxyfen to aquatic environments, it is important to compare the reported toxicity data for nontarget species to that cited as effective against *Ae. aegypti* (≤1 ppb). Generally, pyriproxyfen is many-fold more effective against *Ae. aegypti* than against nontarget organisms giving a clear delineation between the doses required for mosquito control and those resulting in undesirable effects on other species.

The existing field studies that have monitored environmental and nontarget risk have been highly favorable to pyriproxyfen. Schaefer et al. (1991) reported that pyriproxyfen readily adsorbed to organic matter and that it decayed at an exponential rate over a 2-month period. In leaching trials, it was clear that pyriproxyfen did not pose any great environmental risk as the majority of the compound remained locked in the upper 6 cm of a soil column with no great potential for downward migration. Schaefer et al. (1988) stated that after field tests with pyriproxyfen (using 0.11 kg [AI]/ha; an application rate 20 times greater than that needed for control of *Ae. nigromaculis* (Ludlow)), there was minimal environmental risk posed. They monitored effects on the fish *Lepomis macrochirus* (Rafinesque) and *Ictalurus punctatus* (Rafinesque), and a range of invertebrates (Hydra, Cladocera, Copepoda, Ostracoda, Odonata, Dystiscidae, Hydrophilidae, Chironomidae, and Ceratopogonidae). In a separate study, Schaefer and Miura (1990) stated that pyriproxyfen, when used at the above-mentioned rate in rice fields, was "highly compatible with other organisms present in mosquito breeding habitats." There were no detectable residues after 2 d in treated water (<0.01 ppb), no residues in fish (*L. macrochirus*) after 3 d (<5 ppb), and no residues in rice plants (<5 ppb). Slight aberrations in Odonata adults and minor reproductive suppression of cladocerans and ostracods were noted, but they concluded that at the concentrations applied, it was "safe to aquatic, nontarget organisms including mosquito predators."

Brown et al. (1996) noted LC_{50} values of ≈100 ppb against *Leander tenuicornis* (Say), an estuarine shrimp and indicator species. They stated that this was 12 times the estimated field concentration needed for the control of salt marsh mosquitoes in Queensland, Australia. Nuisance chironomid larvae were prevented from emerging from water treated at 10 ppb (Trayler et al. 1994). The EPA (2000) reported EC_{50} values of 56 ppb for green algae and 80–400 ppb for a variety of water fleas (*Daphnia* spp). They also reported that LC_{50} values for bluegill fish and rainbow trout lie between 320 and 5,900 ppb.

Brown et al. (2002) noted that pulse exposures of pyriproxyfen were nontoxic to rainbow fish (*Melanotaenia duboulayi* (Castelnau); common to Australia's freshwater habitats) at 12.5 times the estimated environmental concentrations (EECs) that would be applicable were pyriproxyfen to be applied as a mosquitocide. This was in stark contrast to findings for

temephos. They recommended that pyriproxyfen be used as a larvicide in sensitive habitats containing juvenile fish. In tests on the Pacific blue-eye (*Pseudomugil signifer* (Kner); a larviverous fish abundant in estuarine and freshwater habitats in Australia), pyriproxyfen was toxic at 854 ppb or ≈100 times the EEC were it to be used as a larvicide in these environments. Again, they noted that it was preferable to temephos.

In conclusion, pyriproxyfen is a highly effective larvicide with the potential to be an excellent intervention against dengue (and other *Ae. aegypti*-vectored diseases). It has a very favorable mammalian toxicity profile that suggests that it would be difficult to abuse by misapplication or accidental ingestion. This suggests that it might be suitable for broadcast in the field or at least in gardens and yards without harming humans or other mammals. In Iquitos, the majority of pupal production takes place in passively rain-filled containers outside the house. The sheer profusion of these container types makes them a particularly difficult target to treat (Morrison et al. 2004). The difference in magnitude between the laboratory-derived LC_{50} data for *Ae. aegypti* and that of other nontarget species suggests that it might be applied to such sensitive environments without affecting other species. More work could be conducted to establish optimal field rates with the express purpose of increasing the safety margins.

Finally, it is clear from our data on repellency and on the horizontal transfer of pyriproxyfen that it might be possible to exploit adults as vehicles for the dissemination of this compound. Resting traps like those suggested by Itoh (1993) or oviposition traps such as those proposed by Chism and Apperson (2003) might be used to expose adults and act as a dissemination source. Such transfer methods might be of particular interest for the control of species such as anophelines whose breeding sites are difficult to find but that include discrete volumes of water (e.g., artificial containers and tree boles). These sites might lend themselves to the accumulation of larvicidal doses of pyriproxyfen.

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